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ORIGINAL ARTICLE

Microstructure and textural properties of Kareish cheese manufactured by various ways

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KEYWORDS

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Abstract Kareish cheese was made from skim buffaloes' milk. The effect of the way used in milk coagulation on the microstructural (monitored using scanning electron microscopy) and textural (measured by Emperor ™Lite) properties, as well as chemical, bacteriological and sensory characteristics of the resultant fresh Kareish cheese was studied. Four treatments of Kareish cheese were made by applying coagulation starters of CH-1 (containing of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) (T1) and ABT-1 (containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria) (T2), in addition to use the previous starters with rennet (T3 and T4, respectively) during the cheese-making. Significant differences ($p \leqslant 0.05$) between cheese treatments affected by the type of starter and way used in milk coagulation were observed. Kareish cheese made with starter and rennet (T3) and (T4) was characterised by low contents of fat in dry matter, total protein, ash and pH values, as well as low counts of bacterial starter cells, but high contents of moisture and salt in water phase; as compared with cheeses with starter only (T1 and T2). Also, starter cells of *Str. thermophilus* were prevalent in all cheese treatments, followed by cells of *Lb. delbrueckii* ssp. *bulgaricus* (T1 and T3), *Lb. acidophilus* and bifidobacteria (T2 and T4), respectively. The textural characteristics were revealed that the hardness and gumminess negatively correlated to cohesiveness and springiness. Kareish cheese made with starter and rennet (T3 and T4) had the lowest hardness, gumminess and chewiness values, but the highest values of cohesiveness and springiness. Micrographs were shown contained (T1) and (T2) cheeses on cluster bacterial starter cells. Also, protein aggregates and voids were observed in micrographs of (T3) and (T4) cheeses made with rennet and different starter strains (not shown). Sensory evaluation showed that Kareish cheeses made with different starters and rennet were more accepted by the panellists than with starters only.

Introduction

Kareish is the most popular soft cheese in Egypt. It is acid cheese made from skimmed cow's and buffalo's milk or buttermilk from sour cream, apparently made only on farmsteads.

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Initially, it is made from Laban Khad (*i.e.* fermented buttermilk) or from sour defatted milk, Laban Rayeb. The latter is prepared from fresh whole milk placed in earthenware jars and left undisturbed, the fat rises to the surface and the partly skimmed milk beneath sours. After 24–36 h, the cream layer is skimmed off and the clotted skimmed milk (Laban Rayeb) is poured on to reed mats or into small cheese moulds. After a few hours, the ends of the mat are tied and some whey squeezed out. The pressed curd is permitted to drain further and the squeezing process repeated until the desired texture is obtained; the cured is then cut into pieces and salted. Increased demand has led to the commercial production of Kareish cheese which, under such conditions, is frequently made from pasteurised and/or homogenised milk or reconstituted milk using *Lactobacillus delbrueckii* ssp. *bulgaricus* as starter and usually with rennet rather than acid as coagulant (Phelan et al., 1993).

Cheese texture may be defined as a composite of sensory attributes resulting from a combination of physical properties perceived by the sense of sight and touch (Fox et al., 2000). The rheology of cheese is a function of its composition, microstructure (*i.e.* the structural arrangement of the components), the physicochemical state of its components such as the ratio of solid fat to liquid fat, and its microstructure, which reflects the presence of heterogeneities such as curd granule junctions, cracks and fissures (Gunasekaran and Ak, 2003). A number of factors, both compositional and process parameters, are known to influence texture of cheese (Wium et al., 2003). Milk coagulation, the initial step in the manufacturing of many dairy products (Castillo et al., 2006) including cheese, can be expected to determine the structural organisation of the components in cheese gel (Madadlou et al., 2006).

Rennet is used in cheese making and is important in the formation of the casein network during coagulation (Prasad and Alvarez, 1999). In rennet-induced curds, coagulation phenomenon is greatly influenced by type of rennet (Esteves et al., 2003), concentration of rennet (Madadlou et al., 2005), temperature (Esteves et al., 2003), and time of coagulation (Wium et al., 2003), among other factors. Before they are renneted, the casein micelles in milk show no tendency to aggregate (Dalgleish, 1997). When milk is renneted, casein micelles aggregate (Walstra, 1999), providing a clear transition from a stable dispersion to a flocculated and gelled preparation (De Kruif, 1999). One may expect improvement of the textural and functional characteristics of low-fat cheese by the addition of higher rennet during cheese making, which leads to the higher retention of active rennet in the cheese curd (Madadlou et al., 2006).

The use of starter cultures containing lactic acid bacteria is an essential requirement in the manufacture of most cheeses (Cogan and Hill, 1993) including Kareish cheese. Their major function is to produce lactic acid and, in some cases, flavour compounds (Fox et al., 2000). It is well known that reduction in milk pH due to acidification by starter cells or any other factor is accompanied by micellar demineralization (Banon and Hardy, 1992), which affects cheese curd strength (Khosroshahi et al., 2006). *Lactobacillus* (*Lb.*) and *Bifidobacterium* (*B.*) are the most common species of bacteria used as probiotics for the production of fermented milks and other dairy products (Fuller, 1992). Fresh cheese appears to be ideally suited to serve as a carrier for probiotic bacteria as it is an unripened cheese. During storage it is submitted to refrigeration temperatures and its shelf life is rather limited (Heller et al., 2003). So, many researches were incorporated different probiotic or/and prebiotics during manufacturing to produce functional dairy products, such as soft cheeses (Effat et al., 2012). Also, freeze-dried concentrates of *Bifidobacterium infantis* have been used to produce a cultured cottage cheese (Blanchette et al., 1996).

In recent years, much attention has been given to the microstructure of cheese. Several techniques have been used for this purpose. In particular, the use of scanning electron microscopy has become the method of choice in many investigations (Madadlou et al., 2005, 2006, 2007; Khosroshahi et al., 2006) and it has proved to be an efficacious method to identify cheese components when fat, protein, and moisture are the major constituents (Rahimi et al., 2007). Kalab and Harwalkar (1974) found that skim milk gel containing 60% solids had fused casein micelles within its microstructure and was firm whereas gel with only 40% solids had fewer fused micelles and lacked firmness. Chemicals, such as $CaC1_2$, which increased the firmness of milk gel, also caused casein micelles to fuse (Lee and Marshall, 1981). Very limited data exist in the literature regarding the use of electron microscopy (scanning or transmission) to evaluate the microstructure of Kareish cheese.

The objective of this study was to evaluate the effect of the way used in milk coagulation on the microstructural (monitored using scanning electron microscopy) and textural (measured by Emperor ™Lite) properties, as well as chemical, bacteriological and sensory characteristics of the resultant fresh Kareish cheese.

Materials and methods

Materials

Fresh raw buffaloes' milk was obtained from the herd of the Faculty of Agriculture, Ain Shams University. Buffalos' milk was mechanically separated to skim milk (0.1% fat and 9.5% SNF) for manufacturing Kareish cheese. Commercial fine grade salt of El-Nasr Salines Company, Egypt and calcium chloride from Sigma Chemical Company, Str. Louis, USA, were used.

As coagulant, Hannilase rennet powder (CHY-Max powder extra) was purchased from Chr. Hansen's Lab., Denmark. Two commercial freeze-dried DVS mixed bacterial starters of CH-1 (containing of *Lb. delbrueckii* ssp. *bulgaricus* and *Streptococcus* (*Str.*) *thermophilus*) as yoghurt starter and ABT-1 (containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria) with potential probiotic properties (from Chr. Hansen Laboratory, Copenhagen, Denmark) were used in the fermentation process. Freeze-dried bacterial starters were prepared separately as mother cultures in autoclaved (121 °C/10 min) fresh buffaloes' skim milk using 0.02% (w/v) inoculums. The cultures were incubated at 42 °C for CH-1 starter and 39 °C for ABT-1 starter, until curdling of milk. Cultures (10^8–10^9 cfu/ml) were prepared 24 h before use.

Methods

Kareish cheese manufacture

Four pilot-scale batches of Kareish cheese were made as described by Hussein (1994). Four equal portions of skim milk

was heat treated at 72 °C for 15 s then cooled to 39–40 °C. The first and second portions were inoculated with 3% (v/v) of CH-1 (T1) and ABT-1 (T2) mother cultures, respectively. Other two portions were supplemented with $CaC1_2$ (0.02%, v/v of milk), inoculated with 3% of CH-1 (T3) and ABT-1 (T4) cultures and renneted (3 g/100 kg of milk) was added. All skim milk treatments were stirred well, and held until to coagulate. Sodium chloride (0.5%, w/v of milk) was added between cheese layers and left to whey drain into small cheese moulds at room temperature for ~4–5 h. Cheese blocks were monitored to different analyses after overnight storage at 5 ± 1 °C.

Chemical analyses

Moisture, fat (using Gerber method), total nitrogen (using micro-Kjeldahl method), salt and ash (using Thermolyne, Type 1500 Muffle Furnace) contents; as well as pH values (using pH meter, Hanna Instruments, Italy Srl) were determined in milk and Kareish cheese treatments according to the methods described in AOAC (2000). The fat in dry matter (FDM) and salt in water phase (SWP) were also calculated for all samples analysed.

Bacteriological analyses

Samples of all Kareish cheese were prepared for bacteriological analysis according to the method described in the Standard Methods for the Examination of Dairy Products (Wehr and Frank, 2004). Viable cell counts of *Lb. delbrueckii* ssp. *bulgaricus* on MRS agar (pH 5.2, anaerobic incubation at 45 °C for 72 h), *Lb. acidophilus* on MRS-sorbitol agar (Anaerobic incubation at 37 °C for 72 h), *Str. thermophilus* on ST agar (Aerobic incubation at 37 °C for 24 h) and bifidobacteria on MRS agar (Oxoid) supplemented with L-cystein and lithium chloride (Sigma Chemical CO., USA) (Anaerobic incubation at 37 °C for 72 h) were enumerated as described by Dave and Shah (1996). The plates were incubated in an anaerobic environment (BBL Gas Pak, Becton Dickinson Microbiology Systems). The results expressed as $\log_{10}$ colony forming unit (cfu)/g of sample.

Textural measurements

Force and torque measurements of Kareish cheese treatments were measured using a Texturometer model Mecmesin Emperor ™Lite 1.17(USA). Mechanical primary characteristics of hardness, springiness, gumminess and cohesiveness were determined from the deformation Emperor ™Lite Graph. Also the secondary characteristic of chewiness (hardness × cohesiveness × springiness) was selected because the Kareish cheese showed springiness (Lobato-Calleros et al., 1997).

Microstructure determination

Different fresh Kareish cheese blocks (~5–6 mm^3) were prepared for scanning electron microscopy (SEM) following the method of Brooker and Wells (1984). Samples were viewed by SEM (JXA-840A Electron Probe Microanalyzer-JEOL, Japan) after dehydration using Critical Point Dried instrument and coating with gold using S150A Sputter Coater-Edwards, England.

Sensory analyses

Organoleptic evaluation was carried out according to the scheme of Bodyfelt and Potter (2009). Kareish cheese samples were subjected to organoleptic analyses by 10 staff members of the Food Science Department (Fac. Agric., Ain Shams Univ., Cairo, Egypt). The sensory attributes evaluated were: The flavour (1–10 points), body and texture (1–5 points) and appearance and colour (1–5 points).

Statistical analyses

All experiments and analyses were done in triplicate. All statistical analyses were carried out using the SPSS 16.0 Syntax Reference Guide (SPSS, 2007). The results were expressed as least squares means with standard errors of the mean. Statistically different groups were determined by the LSD (least significant difference) test ($p \leqslant 0.05$).

Results and discussion

Chemical properties

Chemical composition of fresh Kareish cheese manufactured by various ways is presented in Table 1. The results revealed that, the way used in milk coagulation was affected on the chemical composition ($p \leqslant 0.05$) of the resultant cheese. Kareish cheese made with yoghurt starter (T1) showed a decrease in moisture and SWP contents and pH values, but an increase in FDM and total protein (TP) contents, than cheese with probiotic starter (T2). Also, Kareish cheese made with starter and rennet (T3 and T4) was characterised by low contents of FDM, TP, ash and pH values, but high contents of moisture and SWP, as compared with cheeses with starter only (T1 and T2). These results were in agreed with the result obtained by Korish and Abd-Elhamid (2012). Also, Effat et al. (2001)

Table 1 Chemical composition of fresh Kareish cheese manufactured by various ways.

Cheese treatments	Moisture (%)	Fat in dry matter (%)	Total protein ($N \times 6.38$) (%)	Salt in water phase (%)	Ash (%)	pH
T1	70.22 ± 0.10	0.31 ± 0.02	13.96 ± 0.10	0.38 ± 0.10	2.17 ± 0.10	4.67 ± 0.03
T2	71.86 ± 0.15	0.30 ± 0.02	13.19 ± 0.15	0.39 ± 0.20	2.05 ± 0.15	5.08 ± 0.05
T3	72.17 ± 0.15	0.29 ± 0.04	13.05 ± 0.15	0.40 ± 0.15	2.02 ± 0.20	5.17 ± 0.02
T4	72.88 ± 0.20	0.28 ± 0.05	12.71 ± 0.20	0.41 ± 0.15	1.99 ± 0.20	5.28 ± 0.04

T1 = Kareish cheese made with starter culture containing of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus*.
T2 = Kareish cheese made with starter culture containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria.
T3 = as T1 + rennet.
T4 = as T2 + rennet.

Table 2 Viable cell counts ($\log_{10}$ cfu[a]/ml) of bacterial starter strains in fresh Kareish cheese manufactured by various ways.

Cheese treatments	Type of starter				
	CH-1		ABT-1		
	Lb. delbrueckii ssp. bulgaricus	*Str. thermophilus*	*Lb. acidophilus*	*Str. thermophilus*	Bifidobacteria
T1	7.8 ± 0.05	8.8 ± 0.04	–	–	–
T2	–	–	7.6 ± 0.05	8.7 ± 0.06	7.3 ± 0.07
T3	7.4 ± 0.05	8.1 ± 0.04	–	–	–
T4	–	–	7.3 ± 0.05	8.2 ± 0.06	7.1 ± 0.07

T1 = Kareish cheese made with starter culture containing of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus*.
T2 = Kareish cheese made with starter culture containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria.
T3 = as T1 + rennet.
T4 = as T2 + rennet.
[a] Colony forming unit.

Table 3 Textural characterises of fresh Kareish cheese manufactured by various ways.

Cheese treatments	Hardness (N)	Springiness (mm)	Gumminess (N)	Cohesiveness	Chewiness (N/mm)
T1	5.5 ± 0.51	0.543 ± 0.211	2.233 ± 0.443	0.400 ± 0.121	1.195 ± 0.257
T2	4.8 ± 0.55	0.555 ± 0.234	2.030 ± 0.456	0.407 ± 0.132	1.084 ± 0.273
T3	4.2 ± 0.42	0.569 ± 0.200	1.718 ± 0.423	0.409 ± 0.115	0.977 ± 0.246
T4	4.0 ± 0.45	0.575 ± 0.210	1.636 ± 0.435	0.413 ± 0.119	0.949 ± 0.254

T1 = Kareish cheese made with starter culture containing of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus*.
T2 = Kareish cheese made with starter culture containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria.
T3 = as T1 + rennet.
T4 = as T2 + rennet.

mentioned that the use of different bacterial strains resulted in a slight effect on moisture content of Kareish cheese. This might be due to the activity of mixed strains for producing acidity.

Bacteriological properties

Significant differences ($p \leqslant 0.05$) were found in log bacterial cell counts between Kareish cheese, as affected by the type of starter and way used in milk coagulation (Table 2). Generally, Kareish cheese made with starter and rennet (T3 and T4) was characterised by low counts of bacterial starter cells, than those cheeses made with starter only (T1 and T2). Also, cells of *Str. thermophilus* were prevalent in all cheese treatments, followed by *Lb. delbrueckii* ssp. *bulgaricus* cells (T1 and T3). On the other hand, count of *Lb. acidophilus* was higher than that of bifidobacteria in (T2) and (T4). These findings are in harmony with those obtained by Abou Dawood (2002), who mentioned that the non-capsulated bifidobacteria were present in fresh Kareish cheese at the level of >6.36 log cfu/g. Also, Abd-Elhamid (2012) observed that the number of free *Bifidobacterium adolescentis* ATCC 15704 in fresh Kareish cheese present was 7.96 log cfu/g.

Textural characterises

Textural characterises of fresh Kareish cheese manufactured by various ways are observed in Table 3. Differences between treatments ($p \leqslant 0.05$) were affected by the type of starter and way used in milk coagulation. Kareish cheese made with starter and rennet (T3 and T4) had the lowest hardness, gumminess and chewiness values, but the highest cohesiveness and springiness values. As compared between two types of starter used, Kareish cheese made with yoghurt starter only (T1) had higher values of hardness, gumminess and chewiness, but lower values of cohesiveness and springiness, as compared with cheese with probiotic starter (T2). The instrumental textural characteristics of hardness and adhesiveness were negatively correlated to cohesiveness and springiness (Lobato-Calleros et al., 1997, 1998). Korish and Abd-Elhamid (2012) mentioned that the lowest values of hardness, springiness and chewiness in Kareish cheese, may be due to the increase in cheese moisture content. Also, Olson and Johnson (1990) indicated that relative amounts of water, protein, and fat were the dominant factors electing cheese hardness. Fat and moisture act as the filler in the casein matrix of cheese texture (Madadlou et al., 2005), giving it lubricity and softness. Increasing the concentration of starter inoculated to milk also decreased cheese fracture stress and made the cheese body weaker. Also, the reduction in the amount of calcium associated with casein molecules would, however, increase electrostatic repulsion between caseins (Lucey et al., 2003) and cause a weakening of the structural bonds (Horne, 1998). This probably took a part in the decrease in value of stress at fracture as the pH at renneting decreased. The obtained results of hardness are in accordance with that of Thomas (1970) who stated that with low pH of the processed cheese, the protein aggregates and firmness increased. Also, Awad et al. (2002) found that the hardness increased with decreasing the pH in block processed cheese. On the other hand, Kaminarides et al. (2006) reported that with increasing the salt and ash contents in blend Halloumi cheese, the hardness of the resulting processed cheese increased.

Cheese microstructure

Microstructures of fresh Kareish cheese manufactured by various ways were examined by electron microscopy (EM) (Fig. 1). Differences between cheeses could be visually observed in images. Micrographs were shown contained cheeses on cluster cells of *Lb. delbrueckii* ssp. *bulgaricus* (*Lb.*) and *Str. thermophilus* (*Str.*) (T1), as well as *Lb. acidophilus* (*La.*), *Str. thermophilus* (*Str.*) and bifidobacteria (b.) (T2). Also, two distinct structures, protein aggregates (P) and voids (V) were observed in EM micrographs of different treatments. Moreover, uniform protein aggregate networks and voids were observed in (T3) and (T4) cheeses made with rennet and different starter strains (not shown), may be due to embed in the protein matrix. Micrographs of cheeses (T3) and (T4) looked not similar in size of the casein clusters and protein aggregates. Cheese structures were appeared like sponge. The protein matrix was continuous microstructures with close network densities. Abou Dawood (2002) reported that EM of non-capsulated bifidobacteria was present in Kareish cheese as free or exposed cells. Kareish cheese made with yoghurt starter culture appeared to have a compact structure (Hofi et al., 2004). Moreover, a compact protein matrix in the microstructure of low fat Iranian White cheese has been related to the extensive elastic character (Madadlou et al., 2006). Every cheese variety has its characteristic structural features that reflect the chemical and biological changes in the cheese (Abd El-Salam and El-Shibiny, 1973). The number of milk fat globules decreased and the protein matrix became more compact (Rahimi et al., 2007). This probably explained the hard texture observed with the low-fat cheeses even though they were significantly higher in moisture content (Bryant et al., 1995). Also, tripling the rennet concentration resulted in a coarser and more compact protein network.

Sensory properties

The scores for sensory evaluation of fresh Kareish cheese manufactured by various ways are presented in Table 4. Significant differences ($p \leqslant 0.05$) were found between cheeses. Where the type of starter and way used in milk coagulation were the principle factors influencing the sensory properties of cheeses prepared. Kareish cheeses made with probiotic starter (T2 and T4) were more accepted by the panellists (reach flavour and creamy body and texture), as compared with cheese made by yoghurt starter (T1 and T3) characterised by slight acid flavour

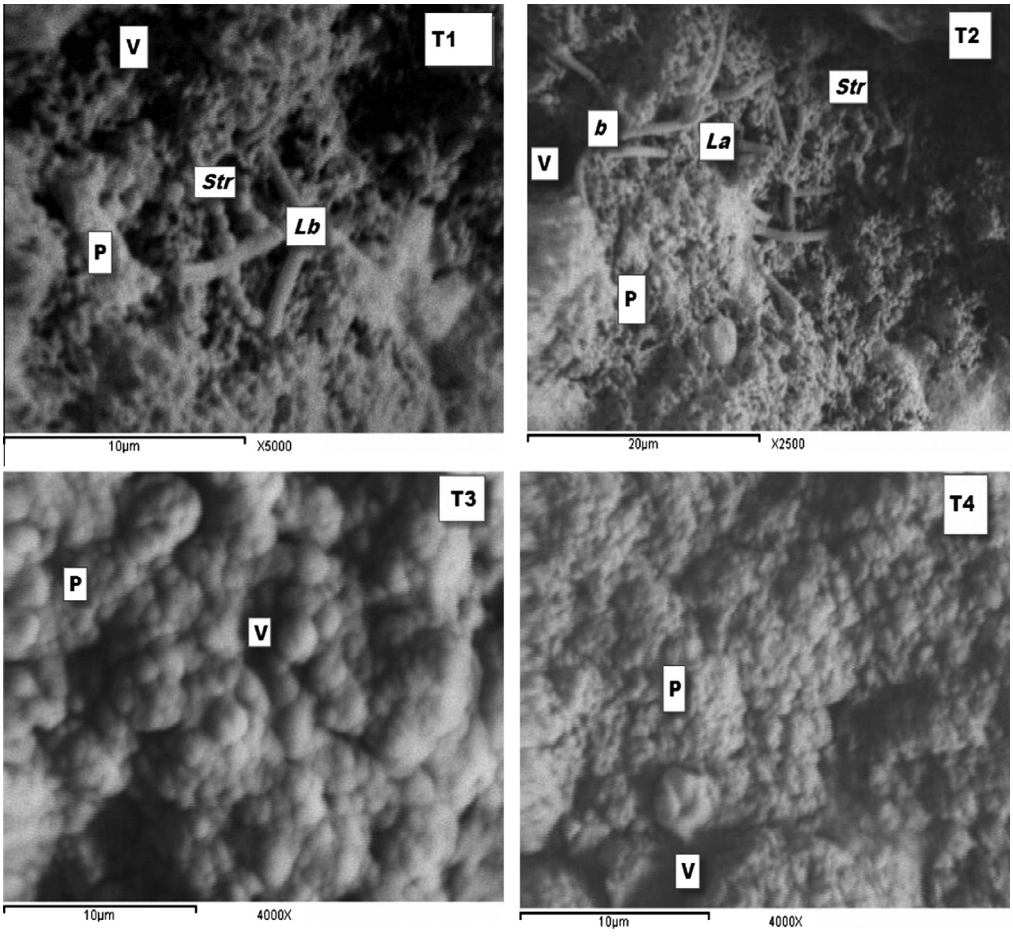

Fig. 1 Scanning electron microscopy of fresh Kareish cheese manufactured by various ways. P: Protein aggregate, V: void, *Lb.*: *Lb. delbrueckii* ssp. *bulgaricus*, *La.*: *L. acidophilus*, *Str.*: *Str. thermophilus* and b.: bifidobacteria. T1 = Kareish cheese made with starter culture containing of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus*. T2 = Kareish cheese made with starter culture containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria. T3 = as T1 + rennet. T4 = as T2 + rennet.

Table 4 Sensory evaluation scores of fresh Kareish cheese manufactured by various ways.

Cheese treatments	Flavour (1–10 points)	Body and texture (1–5 points)	Appearance and colour (1–5 points)	Total (20)
T1	8.3 ± 0.2	4.5 ± 0.1	4.6 ± 0.1	17.4 ± 0.1
T2	10.0 ± 0.0	4.0 ± 0.3	4.3 ± 0.2	18.3 ± 0.2
T3	9.0 ± 0.1	5.0 ± 0.0	4.7 ± 0.1	18.7 ± 0.1
T4	9.7 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	18.9 ± 0.2

T1 = Kareish cheese made with starter culture containing of *Lb. delbrueckii* ssp. *bulgaricus* and *Str. thermophilus*.
T2 = Kareish cheese made with starter culture containing of *Lb. acidophilus*, *Str. thermophilus* and bifidobacteria.
T3 = as T1 + rennet.
T4 = as T2 + rennet.

and cohesive body and texture. Generally, Kareish cheeses made with different starters and rennet had got the highest scores in body and texture (smooth and compact) which could be attributed to rennet added. Moreover, cheeses (T1) and (T3) were characterised by whiter colour than (T2) and (T4). The use of the starter culture in cheese manufacture resulted in some improvements in flavour and aroma development (Hayaloglu et al., 2005). Also, Gobbetti et al. (1998) mentioned that the flavour intensity score of cheeses that had *Bifidobacterium bifidum* and *Bifidobacterium longum* which added individually was slightly higher than that of the Crescenza cheese produced by conventional method, which was probably due to the combination of the higher concentrations of lactic and acetic acids and of free amino acids and soluble peptides.

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